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1 **Title:** Novel measures of inflammation and insulin resistance are related to obesity and
2 fitness in a diverse sample of 11-14 year-olds: The HEALTHY Study
3
4 **Running title:** Fitness, BMI, GlycA and LP-IR in US youth
5
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19 **Conflict of interest:** JDO is an employee of LabCorp, a commercial supplier of NMR-based
20 diagnostic testing. JBB has been a consultant to LipoScience and Quest Diagnostics under a
21 service agreement with his employer. This provides no direct financial benefit to him.
22
23 **Word count = 3624 words**

24 **ABSTRACT**

25 **Background:** GlycA is a novel serum marker of systemic inflammation. There is no
26 information on GlycA in pediatric populations, how it differs by gender or its association
27 with body mass index (BMI) or fitness. LP-IR is a serum measure of insulin resistance which
28 is related to changes in BMI group in adolescents, but its relationship with fitness is
29 unknown. The current study examined the independent associations between fitness and BMI
30 with GlycA and LP-IR among US adolescents.

31 **Methods:** Participants were 1664 US adolescents from the HEALTHY study with complete
32 6th and 8th grade BMI, fitness and blood data. GlycA and LP-IR were measured by NMR
33 spectroscopy. Three BMI groups and three fitness groups were created. Linear mixed models
34 examined associations between GlycA, LP-IR, fitness and BMI.

35 **Results:** LP-IR decreased between 6th and 8th grade. GlycA increased among girls but
36 decreased among boys. At 8th grade, median GlycA values were 27 (7.6%) $\mu\text{mol/L}$ higher
37 (381 versus 354) for girls than boys. Median GlycA 6th grade values were 9% higher in obese
38 girls than healthy weight girls. Overall there was strong evidence ($p < 0.001$) that GlycA was
39 higher in higher BMI groups. Fitness was negatively associated with GlycA ($r = -0.37$ and -
40 0.35) and LP-IR ($r = -0.34$ and -0.18) at the 6th and 8th grade assessments. As BMI category
41 increased and fitness category decreased, GlycA and LP-IR levels increased. Lowest GlycA
42 was found in the low BMI / high fitness group.

43 **Conclusions:** GlycA was associated with BMI and fitness among in US adolescents. These
44 findings suggest that there are independent effects for BMI and fitness group with both
45 GlycA and LP-IR. Future studies should validate the role of GlycA and LP-IR to evaluate the
46 effects of interventions to modify obesity and fitness in order to improve systemic
47 inflammation and insulin resistance.

48

49 INTRODUCTION

50 Obesity and low levels of cardio-respiratory fitness (fitness) are associated with the
51 development of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM)¹⁻³. The
52 physiological mechanisms that contribute to these well established associations are
53 dyslipidemia, insulin resistance and inflammation all of which are associated with obesity
54 and low fitness⁴⁻⁷. A number of studies have demonstrated the adverse effects of increased
55 body fat and low fitness in childhood on future health, with childhood obesity being strongly
56 associated with its persistence into adulthood and fitness tracking through childhood into the
57 adulthood⁸⁻¹⁰. A number of adult studies have suggested that there are independent
58 associations between fitness and body mass index in relation to the risk of cardiovascular
59 disease, type 2 diabetes and all-cause mortality^{1,2}. C-reactive protein (CRP), a marker of
60 systemic inflammation and predictor of cardiovascular risk, has been shown to be closely
61 associated with obesity among children and adults¹¹. Thus, there is a need to examine how
62 various relevant metabolic markers, such as dyslipidemia, insulin resistance and
63 inflammation are associated with body mass and fitness among children. A key issue is
64 whether there are associations between markers of dyslipidemia, insulin resistance and
65 inflammation with body mass and fitness in pediatric populations. If associations exists these
66 markers could be considered potential targets for the reduction of future cardiovascular
67 disease risk.

68

69 GlycA, a novel composite measure of systemic inflammation, and the lipoprotein insulin
70 resistance index (LP-IR) are promising new clinical biomarkers measured by nuclear
71 magnetic resonance (NMR) spectroscopy^{12,13}. Both are obtained efficiently and
72 inexpensively from the same *NMR LipoProfile* test spectra acquired on automated clinical
73 NMR analyzers to quantify lipoprotein particles for use in CVD risk management¹⁴. Several

74 recent reports have appeared relating these new markers to CVD and T2DM risk in adults ¹⁵,
75 ¹⁶, but no comparable data are available in children and adolescents.

76

77 Clinical interest in GlycA stems partly from its composite nature, reflecting the integrated
78 concentrations and glycosylation states of several of the most abundant acute-phase proteins
79 in serum, and its much lower intra-individual biological variability compared to CRP and
80 other markers of inflammation ¹². As a result, GlycA may provide a more stable measure of
81 low-grade systemic inflammation that responds more consistently to diverse inflammatory
82 stimuli than individual acute-phase reactants such as CRP. In several adult studies, GlycA
83 and CRP were found to have independent associations with incident CVD of comparable
84 strength, with some evidence of complementarity suggesting a possible adjunctive clinical
85 use ¹⁷⁻²⁰. Similar observations were made relating GlycA to prediction of future T2DM in the
86 Women's Health Study and Dutch PREVEND study ^{15, 16}.

87

88 The insulin resistance biomarker, LP-IR, reflects the lipoprotein derangements of insulin
89 resistance and is derived by combining 6 NMR measures of very-low-density lipoprotein
90 (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particle size and
91 subclass concentration ¹³. Each of these subclass and size parameters has been shown
92 individually to be associated with incident T2DM in the Women's Health Study ²¹, and the
93 composite LP-IR score exhibited robust diabetes prediction in a large multi-ethnic cohort of
94 men and women ²².

95

96 We previously obtained *NMR LipoProfile* spectra from a substantial subset of 6th and 8th
97 grade participants in the ethnically diverse HEALTHY trial, initially to characterize the
98 differences between NMR-measured lipoprotein particle numbers and traditional lipid

measures in a large pediatric population²³. In a subsequent report, changes in relative weight group from 6th to 8th grade were related to lipoprotein particle changes associated with risk of CVD and T2DM, as well as alterations in insulin resistance assessed by LP-IR and insulin and glucose measurements²⁴. In this paper, we take advantage of the ability, with newly available software, to extract GlycA values from the same *NMR LipoProfile* dataset to address the absence of information in youth regarding relations of body weight and fitness with GlycA and LP-IR. The aims of this study in an ethnically diverse sample of children were to: a) report on levels of GlycA and the change in GlycA as children move from 6th to 8th grade; b) examine whether BMI group is associated with GlycA in these children; c) determine if fitness was associated with GlycA, LP-IR and traditional lipid panel variables; and d) examine whether fitness and BMI are independently related to GlycA and/or LP-IR.

110

111 **METHODS**

The analyses reported in this paper used information from stored blood from the HEALTHY Study, a National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) cluster randomized controlled trial that aimed to reduce the prevalence of risk factors for type 2 diabetes mellitus among middle school children (6th - 8th grade)^{25, 26}. Details of the study design and results have been reported in a number of publications^{25, 26}. Briefly, the study was conducted in 42 middle schools across the US. In order to participate in the study, schools had to have at least 50% of students eligible for free or reduced-price lunch or belonging to an ethnic minority group at increased risk of type 2 diabetes. The intervention had several components including changes to the physical education and cafeteria programs as well as health education and a school wide social marketing campaign^{25, 27-32}. The study was approved by the Institutional Review Boards at each field center and written parental consent and childhood assent was obtained from all participants³³. The sample for this study is

limited to the 1664 participants who provided parental consent/child assent for ancillary analyses of stored blood, and for whom complete data were available.

Procedures

All measures were assessed at baseline (beginning of 6th grade) and follow-up (end of 8th grade). Pubertal status was self-reported using the Pubertal Development Scale³⁴ and converted to pubertal stage groups consistent with the five pubertal stages outlined by Tanner³⁵. Household education were determined from parental report and gender and race/ethnicity was self-reported. Height and body mass were measured without shoes using the Prospective Enterprises PE-AIM-101 stadiometer and the SECA Corporation Alpha 882 electronic scale. Body Mass Index (kg/m^2) was calculated and converted to an age and gender specific BMI percentile using CDC 2000 criteria³⁶. For descriptive purposes participants with a BMI <85th percentile were classified as healthy weight; while BMI \geq 85th percentile but <95th percentile were classified as overweight and those with BMI \geq 95th percentile were classified as obese.

Cardiorespiratory fitness was assessed using the 20-meter shuttle test (20-MST)^{37, 38} during a Physical Education class. The test required students to run back and forth between two lines set 20 meters apart. The running pace was determined by audio signals emitted from a pre-recorded CD. The test started at 8.5 km/hr and increased by 0.5 km/hr with each subsequent level. The test was completed when the participant was not able to complete the distance at the stipulated pace on two laps.

Fasting blood samples were collected from all participants. Standard lipid profiles including HDL-C were measured by CDC-standardized direct assay at the University of Washington³⁹. LDL-C was calculated using the Friedewald equation⁴⁰. Insulin was measured by a two-site

immuno-enzymometric assay⁴¹. Fasting insulin (performed using a Tosoh 1800 auto-analyzer) and glucose (performed on a Roche P module auto-analyzer by the hexokinase method) were used to calculate the homeostatic model assessment of insulin resistance (HOMA-IR) according to the formula: $\text{Glucose} \times \text{Insulin} / [\mu\text{U/L}] \times 22.5$ ⁴². Lipoprotein particle profiles were measured by NMR spectroscopy with the LipoProfile-3 algorithm at LipoScience, Inc (Raleigh, NC) on frozen EDTA plasma specimens and LP-IR and GlycA were derived using previously published procedures^{12, 13}.

Statistical analysis

Overall, 2367 participants in HEALTHY had samples analyzed by LipoScience. Of these, 703 were excluded from the present analysis, most due to imprecise classification of their race or the lack of either a 6th or 8th grade fitness test. Descriptive statistics, including means, standard deviations, and percentages were calculated for those included and excluded from the analysis. Differences between those included and excluded were tested using a generalized linear mixed model which took into account the sources of variability within and between schools. A similar approach was undertaken for baseline characteristics of the analysis sample to examine gender differences.

Measures in 6th grade, 8th grade and change between 6th and 8th grade are summarized using mean and 95% confidence interval for normally distributed measures (number of laps) and medians and interquartile range (IQR) for parameters not fitting a normal distribution (lipid measures, insulin resistance measures and GlycA) by gender. Median and IQR are also presented for GlycA based on BMI category for normal weight (BMI < 85th percentile), overweight (BMI 85th to 94th percentile) and obese (BMI ≥ 95th percentile) and percentiles were calculated by gender and grade for GlycA.

174

175 Generalized linear mixed models were constructed to examine the association between
176 GlycA and BMI category for 6th grade and 8th grade (adjusting for GlycA in 6th grade),
177 separately for girls and boys taking into account the sources of variability within and between
178 schools. These models were adjusted for race/ethnicity, highest household education,
179 intervention group with the 6th grade models also adjusted for 6th grade Tanner stage and the
180 8th grade models adjusted for 8th grade Tanner stage and 6th grade GlycA value. Spearman
181 correlations between GlycA and lipid measures, insulin resistance, BMI percentile, and
182 fitness (number of laps) were calculated for 6th grade and 8th grade with both genders
183 combined since there were no appreciable differences between the genders. The analysis of
184 the 8th grade data, which is adjusted for 6th grade values, allows us to account for the
185 longitudinal nature of the data.

186

187 Level of fitness was classified into quartiles by grade and gender and then further classified
188 as low, medium or high fitness levels. Low fitness level was defined to be those grouped into
189 the first quartile: 6th grade girls 0-11 laps, 6th grade boys 0-12 laps, 8th grade girls 1-12 laps,
190 8th grade boys 1-17 laps. Medium fitness level was defined to be those grouped into the
191 second or third quartile: 6th grade girls 12-23 laps, 6th grade boys 13-30 laps, 8th grade girls
192 13-26 laps, 8th grade boys 18-44 laps. Finally, high fitness was defined as those grouped into
193 the fourth quartile: 6th grade girls 24-57 laps, 6th grade boys 31-75 laps, 8th grade girls 27-79
194 laps, 8th grade boys 45-103 laps. Adjusted means and standard errors were then computed for
195 GlycA, LP-IR, LDL-P and non-HDL-C within BMI classification (normal, overweight,
196 obese) and fitness level. Levels of GlycA, LP-IR, LDL-P and non-HDL-C were then
197 categorized within the three BMI and three fitness groups to create nine subgroups and these

groups were tabulated by gender at both 6th and 8th grade. To further facilitate understanding, the GlycA and LP-IR values in these subgroups were then presented graphically.

All p-values reported within this paper represent findings associated with secondary outcomes from a large cluster randomized controlled trial and these hypotheses were not pre-specified in the trial design. As such, p-values are provided to help facilitate the interpretation of the data only. SAS 9.3 statistical software (SAS Institute, Cary, NC) was used for analyses.

RESULTS

Descriptive statistics for 6th grade participants are presented overall and by gender in Table 1. The data in Table 1 present strong evidence that boys recorded a higher number of shuttle run laps than the girls (23.1 versus 18.5, $p < 0.001$) and some evidence that boys had a higher BMI percentile (75.4 versus 72.4, $p = 0.0264$) than the girls.

Supplemental Table A provides descriptive information on the participants included and excluded from the analyses. These data provide some evidence ($p = 0.0043$) that there was a difference in the ethnicity of the included versus excluded participants with higher proportions of Hispanic (62.7% vs. 40.4%) and White participants (20.7% vs. 12.2%) in the included sample.

Table 2 provides descriptive information (means and 95% CI or median and inter-quartile range) for fitness, lipids, insulin resistance and GlycA variables at 6th and 8th grade along with the temporal changes (8th - 6th grade value) stratified by gender. The table shows that among both girls and boys non-HDL-C, LDL-C and LP-IR decreased between 6th and 8th

grade while HOMA-IR increased. GlycA increased by 16 $\mu\text{mol/L}$ (3.8% based on medians) among girls but decreased by 6 $\mu\text{mol/L}$ (3.1% based on medians) among the boys. At 8th grade the median GlycA values were 27 (7.6%) $\mu\text{mol/L}$ higher (381 versus 354) for girls than boys. Supplementary Table B provides percentiles of GlycA by grade and gender.

Supplementary Table C provides the medians and inter-quartile ranges for GlycA by BMI group stratified by gender and grade level. The median GlycA value for 6th grade obese girls was 417 $\mu\text{mol/L}$ compared to 351 for healthy weight girls, and as such, GlycA levels are 19% higher in obese girls than healthy weight girls. The data in the table provide strong evidence ($p < 0.001$) that in all sub-groups GlycA is higher in higher BMI groups. Supplementary Table D provides Spearman correlations between all of the variables. The number of shuttle run laps is negatively associated with GlycA ($r = -0.37$ and $r = -0.35$) and LP-IR ($r = -0.34$ and $r = -0.18$) for the 6th and 8th grade associations respectively.

Figure 1 provides a graphical presentation of levels of adjusted GlycA by BMI and fitness categories stratified by gender and 6th or 8th grade. The figure demonstrates that across all four sub-groups there is evidence that as BMI category increases and fitness category decreases levels of GlycA increase. Furthermore, in all four sub-groups the lowest levels of GlycA are in the low BMI / high fitness group with the highest levels in the high BMI / low fitness group. Comparable patterns are also evident for LP-IR levels, which are graphically presented in Figure 2. The data used to create Figures 1 and 2 are available in Supplementary Table E.

DISCUSSION

This study is the first to provide descriptive information on levels of GlycA, a new NMR-derived marker of systemic inflammation, in adolescents. The data presented in this study have shown, in an ethnically diverse sample of adolescents, that GlycA is associated with BMI group with levels of GlycA higher across BMI groups among both boys and girls in the 6th and 8th grades. These data also show that GlycA is inversely correlated with shuttle run laps, a surrogate measure of cardiorespiratory fitness at both 6th and 8th grade. Furthermore, when levels of GlycA were analyzed by the three BMI and three fitness groups there is evidence of a relationship between fitness, BMI group and GlycA with the highest levels of GlycA among obese children in the lowest fitness category. The relationship between fitness, BMI and GlycA was similar for both boys and girls at 6th and 8th grade. A key finding of this study is therefore that GlycA, a new measure of systemic inflammation, is associated with both body mass and fitness in a pediatric population. As such, further examination of GlycA in pediatric populations is warranted to identify associations with future disease risk and the response to changes in fitness and body mass among children.

There was evidence that LP-IR was negatively correlated with the number of shuttle laps run at both 6th and 8th grade. We have previously reported that LP-IR is associated with BMI group and change in BMI group among boys and girls in the same sample²⁴. Thus, in this paper we have extended these findings by showing that there are independent effects of fitness and BMI group on LP-IR with the lowest levels of LP-IR among the low BMI / high fitness group and the highest levels among the high BMI / low fitness group. These patterns were comparable among boys and girls at 6th and 8th grade. These finding therefore suggest that facilitating increased fitness and lower BMI is likely to be important for achieving lower levels of LP-IR among adolescents, which may reduce overall CVD risk.

273

274 The association reported in this paper between fitness, BMI and GlycA is broadly consistent
275 with previous research, which has shown that fitness is associated with CRP, another measure
276 of systemic inflammation, after accounting for body mass. For example, in a cross-sectional
277 analysis, the fitness levels of young adults were inversely associated with CRP and this
278 association was maintained even after adjustment for body mass index ⁴³. Similarly, among
279 adults with type 2 diabetes a change in fitness over 12-months was associated with change in
280 CRP and this association was independent of BMI change ⁴⁴. In adolescents, fitness and
281 fatness (assessed via skinfolds) have been were independently associated with systemic
282 inflammation, as measured by CRP ⁴⁵. There is some evidence that GlycA may be a better
283 marker of shorter-term CVD risk (events occurring within ~6 years) than longer-term risk
284 which may mean that GlycA can serve as an early marker of adult disease risk but it is not
285 currently clear if this is the case for adolescents ¹⁷. However, GlycA has a lower level of
286 intra-individual variability than CRP which might make it attractive as a CVD risk marker
287 across time. As CRP was not available in the HEALTHY study, it is not possible to directly
288 compare the associations between CRP and GlycA, fitness and BMI group in this dataset. As
289 such, we are unable to state that one biomarker may be preferable to another, but such an
290 assessment in a future study is warranted. Moreover, it would be useful to assess whether
291 intervention studies that target increased fitness and reduced body mass might yield
292 improvements in GlycA.

293

294 Higher levels of cardio-respiratory fitness have been associated with a reduced risk of
295 developing cardiovascular disease and type 2 diabetes among adults ^{1,2}. For example, recent
296 analyses of the CARDIA study have shown that fitness in young adulthood was associated
297 with all-cause mortality approximately 27 years later with each additional minute of exercise

test duration associated with a 15% lower hazard of death³. Interestingly the study showed that CRP was much higher in the low fitness group, thereby highlighting the important link between fitness and inflammation. The current study extends this work to show how fitness and obesity are independently associated with GlycA, a novel measure of systemic inflammation in a pediatric population.

GlycA levels were 7% higher for girls than boys at 8th grade but were comparable between the genders in 6th grade. This finding is broadly consistent with the adult literature in which CRP levels are higher among women than men^{46, 47} despite the lack of a difference in absolute or relative risk of cardiovascular events when compared to men⁴⁸. We presume that this gender difference may represent a hormonal influence upon GlycA, which is independent of its role as a CVD risk marker. The emergence of higher levels of GlycA in 8th grade girls, therefore, might be expected with the progression of puberty, generally occurs between 6th and 8th grade in girls. For example, in this study the proportion of girls classified as Tanner Stage 4 or 5 at 6th grade was 23.5% but by 8th grade this had increased to 94.2%. As previous research has shown that the advancement of puberty is associated with changes in insulin sensitivity⁴⁹ it may also be the case that pubertal hormones exert more direct effects on systemic inflammation, and this influence may persist beyond puberty. Further research into hormonal influences on these markers of inflammation is therefore warranted.

Strengths / limitations

The major strength of this study is the provision of a detailed analysis of GlycA and LP-IR in a large, ethnically diverse sample of young people progressing from 6th to 8th grade. No comparable data exist and as such these data make a unique contribution to the field. However, the study has several limitations to be considered. Firstly, a field based measure of

fitness (shuttle run laps) was used in the analyses. Although this measure has been shown to be closely correlated with directly measured oxygen uptake it is less precise than laboratory based methods⁵⁰⁻⁵². Secondly, we used a self-report measure for assignment of pubertal status. Although this measure has been validated, it is not generally considered to be as reliable as clinician assessment of Tanner staging³⁴. Thirdly, the analyses in this paper have focused on GlycA, a new measure of systemic inflammation, but as CRP is not available in this dataset it is not possible to assess how associations may compare to the more widely used marker of systematic inflammation. Fourth, it is important to recognize that the analyses reported in this paper were conducted on a sub-set of participants who provided consent for ancillary analyses and complete data for all variables were available. In particular, the observed difference in the ethnicity of the included versus the excluded participants is a potential weakness. Fifth, it is important to note that consistent with other studies^{1,2} that have examined the association between fitness, body mass and health outcomes we have only assessed and analyzed cardio-vascular fitness and have no data to comment on broader aspects of fitness such as strength. Finally, this is a post hoc analysis of secondary endpoints and our analyses have not been corrected for the multiple comparisons being made, and should therefore be regarded as exploratory.

CONCLUSIONS

GlycA, a new measure of systemic inflammation, was associated with BMI and fitness among an ethnically diverse sample of adolescents in the US. Analyses also provided evidence of independent effects for BMI and fitness groups when related to both GlycA and LP-IR, a multivariate insulin resistance score. These findings suggest that reducing body mass and increasing fitness may reduce both systemic inflammation (GlycA) and insulin resistance (LP-IR). Further examination of how body mass, fitness and changes in both of

these health indicators is associated with GlycA in pediatric populations is therefore warranted.

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Conflict of interest: JDO is an employee of LabCorp, a commercial supplier of NMR-based diagnostic testing. JBB has been a consultant to LipoScience and Quest Diagnostics under a service agreement with his employer. This provides no direct financial benefit to him.

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564 **FIGURE LEGENDS**

565 **Figure 1:** Inflammation (GlycA) in Obesity and Fitness Subgroups

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567 **Figure 2:** Insulin Resistance (LP-IR) in Obesity and Fitness Subgroups

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Table 1: Baseline Characteristics

	Gender						p-value**
	OVERALL		Female (N=897)		Male (N=767)		
	Mean (SD) or		Mean (SD) or		Mean (SD) or		
	N and %		N and %		N and %		
Age (years)	11.28	(0.55)	11.22	(0.50)	11.35	(0.60)	<.0001
Number of Laps	20.63	(11.60)	18.53	(9.33)	23.09	(13.38)	<.0001
BMI Percentile	73.75	(27.48)	72.37	(27.35)	75.35	(27.57)	0.0264
BMI Category							0.0019
< 85 th Percentile	818	49.2%	471	52.5%	347	45.2%	
85 th – 94 th Percentile	331	19.9%	184	20.5%	147	19.2%	
≥ 95 th Percentile	515	30.9%	242	27.0%	273	35.6%	
Race/Ethnicity							0.3107
Hispanic	1044	62.7%	573	63.9%	471	61.4%	
Black	276	16.6%	151	16.8%	125	16.3%	
White	344	20.7%	173	19.3%	171	22.3%	
Positive Reported 1 st Degree Family History of Diabetes	215	12.9%	115	12.8%	100	13.0%	0.9214
Highest Household Education							0.8817
≤ HS Graduate	837	50.3%	473	52.7%	364	47.5%	
≥ Some college	827	49.7%	424	47.3%	403	52.5%	
6 th Grade Pubertal Status							*
Tanner Stage 1	168	10.1%	49	5.5%	119	15.5%	
Tanner Stage 2	436	26.2%	120	13.4%	316	41.2%	
Tanner Stage 3	669	40.2%	379	42.3%	290	37.8%	
Tanner Stage 4	357	21.5%	315	35.1%	42	5.5%	
Tanner Stage 5	34	2.0%	34	3.8%	0	0	

*Test does not converge due to zero cells.

**p-values obtained from generalized linear mixed models taking account of sources of variability within and between schools.

Table 2: 6th Grade, 8th Grade and Change (8th-6th) in Fitness, Lipids, Insulin Resistance and GlycA by Gender

	<i>Female</i>						<i>Male</i>					
	<i>6th Grade</i>		<i>8th Grade</i>		<i>Difference (8th – 6th)</i>		<i>6th Grade</i>		<i>8th Grade</i>		<i>Difference (8th – 6th)</i>	
<i>Fitness (# of laps)</i>	18.5	(17.9, 19.2)	21.2	(20.4, 21.9)	2.7	(2.0, 3.3)	23.1	(22.1, 24.0)	33.9	(32.6, 35.3)	10.8	(9.7, 12.0)
<i>Non-HDL-C (mg/dL)</i>	102	(86, 120)	95	(81, 110)	-7	(-18, 4)	105	(88, 124)	93	(79, 111)	-11	(-23, 1)
<i>LDL-C (mg/dL)</i>	83	(71, 99)	79	(67, 93)	-4	(-15, 5)	88	(73, 104)	77	(64, 93)	-10	(-21, -0)
<i>HDL-C (mg/dL)</i>	51	(44, 59)	53	(45, 62)	2	(-3, 7)	51	(44, 60)	48	(41, 56)	-3	(-8, 2)
<i>HOMA-IR</i>	2.62	(1.76, 4.19)	3.35	(2.34, 4.77)	0.61	(-0.42, 1.74)	2.11	(1.36, 3.36)	3.03	(1.94, 4.62)	0.84	(-0.12, 2.12)
<i>LP-IR</i>	32	(20, 49)	28	(16, 42)	-5	(-15, 5)	38	(22, 58)	36	(22, 54)	-1	(-13, 9)
<i>GlycA (μmol/L)</i>	374	(334, 421)	397	(361, 442)	24	(-16, 58)	379	(333, 425)	365	(322, 418)	-10	(-49, 32)

Non-HDL-C, LDL-C, HDL-C, HOMA-IR, LP-IR and GlycA are presented as medians and (25th percentile, 75th percentile) while fitness is presented as mean and 95% confidence interval.

Figure 1: Inflammation (GlycA) in Obesity and Fitness Subgroups

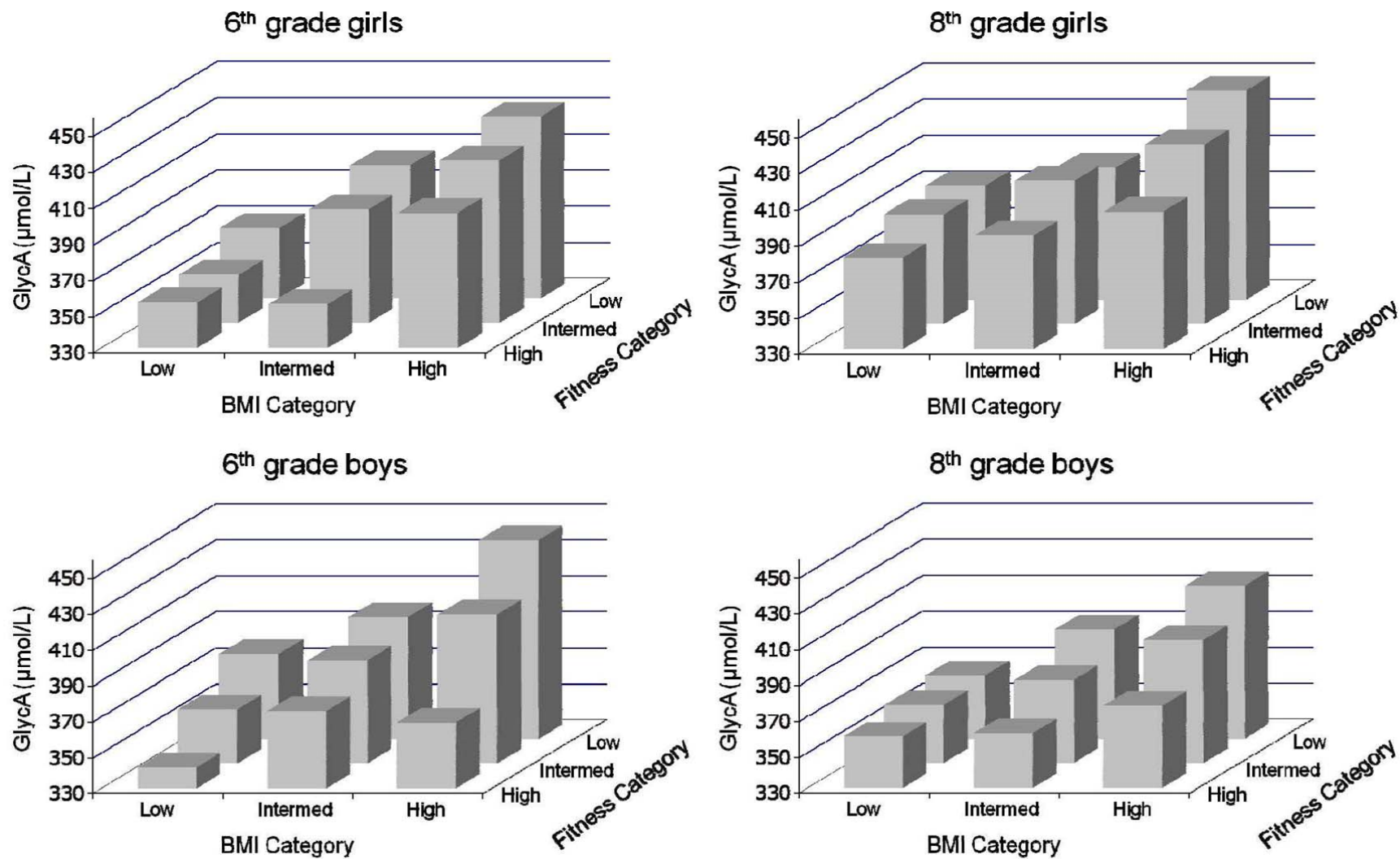


Figure 2: Insulin Resistance (LP-IR) in Obesity and Fitness Subgroups

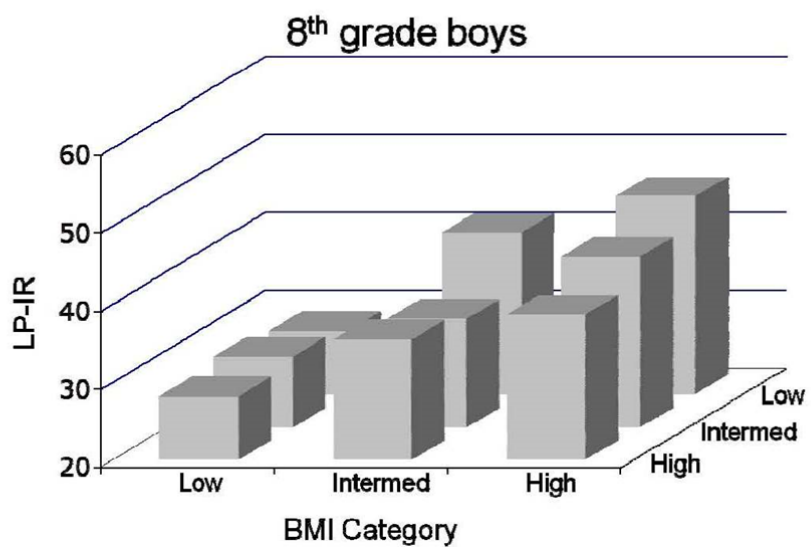
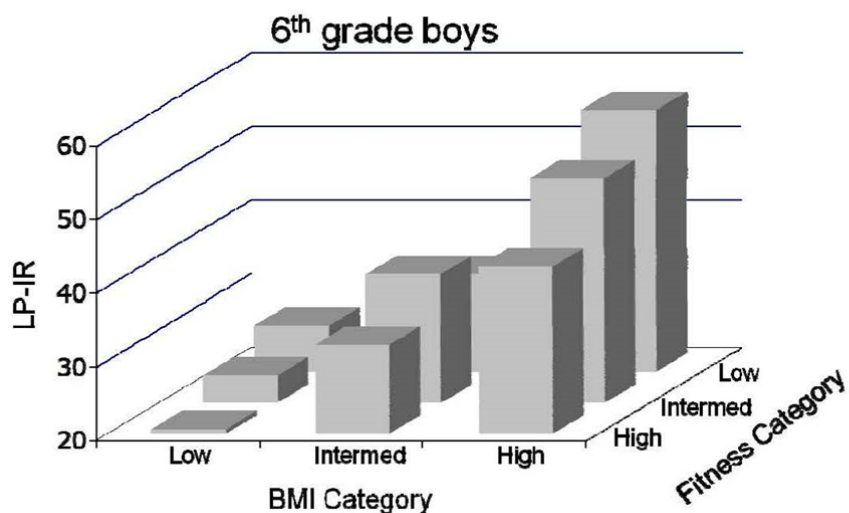
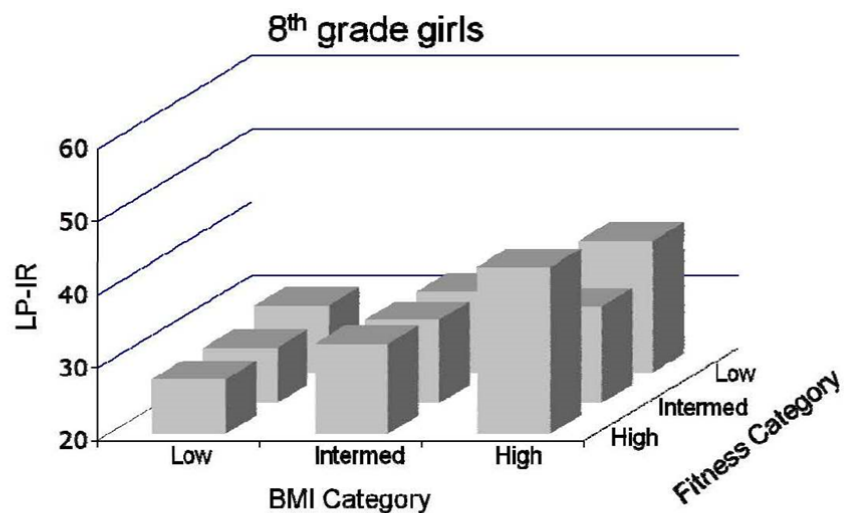
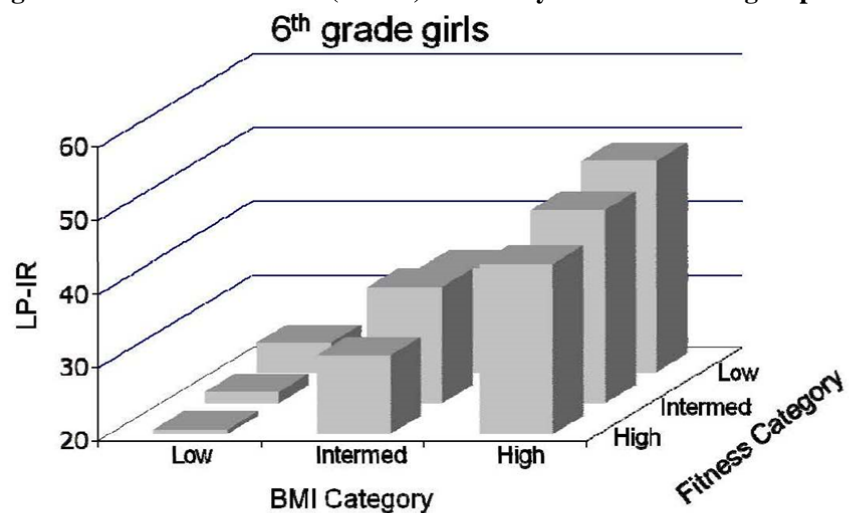


Table A: Comparison of Subjects Included and Excluded from Analysis

	Analysis Status						<i>p</i> -value**
	OVERALL		Included (<i>N</i> =1664)		Excluded (<i>N</i> =703)		
	Mean (SD) or		Mean (SD) or		Mean (SD) or		
	<i>N</i> and %		<i>N</i> and %		<i>N</i> and %		
Age (years)	11.27	(0.57)	11.28	(0.55)	11.26	(0.60)	0.6780
Gender							0.3834
Female	1288	54.4%	897	53.9%	391	55.6%	
Male	1079	45.6%	767	46.1%	312	44.4%	
Number of Laps	20.48	(11.63)	20.63	(11.60)	20.05	(11.72)	0.0617
Randomization Status							0.3756
Control	1229	51.9%	835	50.2%	394	56.0%	
Intervention	1138	48.1%	829	49.8%	309	44.0%	
Baseline BMI Percentile	72.89	(27.98)	73.75	(27.48)	70.86	(29.05)	0.0792
Baseline BMI Category							0.6775
< 85 th Percentile	1183	50.0%	818	49.2%	365	51.9%	
85 th – 94 th Percentile	462	19.5%	331	19.9%	131	18.6%	
≥ 95 th Percentile	722	30.5%	515	30.9%	207	29.4%	
Race/Ethnicity							0.0043*
Hispanic	1328	56.1%	1044	62.7%	284	40.4%	
Black	402	17.0%	276	16.6%	126	17.9%	
White	430	18.2%	344	20.7%	86	12.2%	
Other	207	8.7%	0	0%	207	29.4%	
Highest Household Education							0.4280
≤ HS Graduate	1136	49.1%	837	50.3%	299	46.1%	
≥ Some college	1177	50.9%	827	49.7%	350	53.9%	
Baseline LDL-C (mg/dL)	87.26	(22.68)	87.50	(22.54)	86.70	(23.02)	0.1813
Baseline HDL-C (mg/dL)	52.81	(12.33)	53.52	(12.96)	52.51	(12.05)	0.4280

*Other Race not used for test

**p-values obtained from generalized linear mixed models taking account of sources of variability within and between schools.

Table B: Percentiles of GlycA (μmol/L) by Gender and Grade

	<i>Girls</i>		<i>Boys</i>	
	<i>6th Grade</i>	<i>8th Grade</i>	<i>6th Grade</i>	<i>8th Grade</i>
<i>Minimum</i>	223	251	243	235
<i>1st Percentile</i>	272	283	264	246
<i>5th Percentile</i>	293	309	284	279
<i>10th Percentile</i>	308	330	302	296
<i>25th Percentile</i>	334	361	333	322
<i>50th Percentile</i>	374	397	379	365
<i>75th Percentile</i>	421	442	425	418
<i>90th Percentile</i>	470	487	477	470
<i>95th Percentile</i>	501	519	505	502
<i>99th Percentile</i>	562	599	549	579
<i>Maximum</i>	807	755	591	771

Table C: Medians for GlycA by Weight Category and Gender and Tests for Associations between Category and GlycA

Girls										
	BMI < 85 th Percentile			BMI 85 th – 94 th Percentile			BMI ≥ 95 th Percentile			
	Median	(Q1,	Q3)	Median	(Q1,	Q3)	Median	(Q1,	Q3)	p-value
6 th Grade GlycA* (μmol/L)	351	(317,	392)	386	(347,	424)	417	(386,	464)	<.0001
8 th Grade GlycA** (μmol/L)	379	(344,	417)	407	(367,	444)	443	(403,	488)	<.0001
Boys										
	BMI < 85 th Percentile			BMI 85 th – 94 th Percentile			BMI ≥ 95 th Percentile			
	Median	(Q1,	Q3)	Median	(Q1,	Q3)	Median	(Q1,	Q3)	p-value
6 th Grade GlycA* (μmol/L)	340	(311,	386)	378	(344,	417)	419	(386,	459)	<.0001
8 th Grade GlycA** (μmol/L)	341	(306,	377)	369	(332,	417)	415	(379,	463)	<.0001

*Models adjusted for 6th grade Tanner stage, race/ethnicity, highest household education, and intervention group

**Models adjusted for 8th grade Tanner stage, 6th grade value of the GlycA, race/ethnicity, highest household education, and intervention group

Table D: Spearman Correlations for Between GlycA, Lipids, Lipoproteins, Insulin Resistance, Fitness and BMI (not adjusting for school cluster) by Grade

	6 th Grade							
	<i>LP-IR</i>	<i>HOMA-IR</i>	<i>BMI %ile</i>	<i>HDL-C</i>	<i>LDL-C</i>	<i>LDL-P</i>	<i>Non-HDL-C</i>	<i>Number of Laps</i>
GlycA (μmol/L)	0.41	0.41	0.50	-0.33	0.18	0.39	0.29	-0.37
LP-IR	--	0.49	0.59	-0.69	0.13	0.54	0.37	-0.34
HOMA-IR		--	0.65	-0.41	0.09	0.31	0.24	-0.41
BMI Percentile			--	-0.46	0.18	0.41	0.31	-0.50
HDL-C (mg/dL)				--	-0.03	-0.39	-0.20	0.29
LDL-C (mg/dL)					--	0.68	0.92	-0.10
LDL-P (nmol/L)						--	0.76	-0.28
Non-HDL-C (mg/dL)							--	-0.18
	8 th Grade							
	<i>LP-IR</i>	<i>HOMA-IR</i>	<i>BMI %ile</i>	<i>HDL-C</i>	<i>LDL-C</i>	<i>LDL-P</i>	<i>Non-HDL-C</i>	<i>Number of Laps</i>
GlycA (μmol/L)	0.31	0.42	0.43	-0.24	0.20	0.35	0.28	-0.35
LP-IR	--	0.43	0.47	-0.72	0.09	0.48	0.28	-0.18
HOMA-IR		--	0.55	-0.30	0.12	0.28	0.24	-0.33
BMI Percentile			--	-0.41	0.19	0.38	0.28	-0.37
HDL-C (mg/dL)				--	-0.04	-0.42	-0.16	0.10
LDL-C (mg/dL)					--	0.70	0.94	-0.15
LDL-P (nmol/L)						--	0.76	-0.23
Non-HDL-C (mg/dL)							--	-0.19

Table E: Adjusted mean (SE) level of measures of inflammation, insulin resistance, and atherogenic lipoproteins by obesity and fitness subgroup									
Girls									
6 th Grade									
	BMI <85 th %ile			BMI 85 th – 94 th %ile			BMI ≥95 th %ile		
	Low Fitness	Med Fitness	High Fitness	Low Fitness	Med Fitness	High Fitness	Low Fitness	Med Fitness	High Fitness
GlycA (μmol/L)	369.13 (8.59)	356.81 (5.34)	355.31 (5.49)	403.43 (9.09)	392.67 (6.88)	354.42 (10.55)	430.16 (6.40)	419.56 (6.54)	404.77 (16.55)
LP-IR	23.96 (0.05)	21.50 (0.02)	20.59 (0.02)	34.14 (0.06)	35.94 (0.03)	30.83 (0.08)	48.78 (0.03)	46.27 (0.03)	43.18 (0.20)
LDL-P (nmol/L)	611.98 (33.79)	646.68 (20.76)	602.41 (21.38)	712.57 (35.84)	755.87 (26.95)	664.56 (41.65)	876.62 (25.04)	866.93 (25.59)	776.56 (65.60)
Non-HDL-C (mg/dL)	99.04 (3.40)	101.17 (2.02)	100.05 (2.10)	108.98 (3.63)	111.12 (2.69)	100.59 (4.24)	115.26 (2.49)	117.46 (2.54)	110.24 (6.74)
8 th Grade									
GlycA (μmol/L)	393.05 (11.92)	390.87 (10.67)	381.04 (11.06)	402.69 (13.34)	409.21 (11.35)	392.93 (13.45)	445.79 (11.22)	429.86 (11.21)	406.27 (17.46)
LP-IR	29.23 (0.09)	27.58 (0.07)	27.52 (0.08)	31.12 (0.11)	31.49 (0.08)	32.36 (0.12)	37.97 (0.08)	33.31 (0.08)	42.90 (0.20)
LDL-P (nmol/L)	709.12 (39.57)	718.39 (35.43)	716.93 (36.52)	737.51 (44.60)	759.70 (38.00)	696.38 (44.31)	832.58 (37.67)	731.10 (37.71)	774.29 (58.31)
Non-HDL-C (mg/dL)	100.66 (3.56)	99.76 (3.18)	99.33 (3.29)	99.57 (4.00)	102.59 (3.40)	95.60 (4.01)	104.69 (3.34)	100.15 (3.35)	98.10 (5.21)
Boys									
6 th Grade									
	BMI <85 th %ile			BMI 85 th – 94 th %ile			BMI ≥95 th %ile		
	Low Fitness	Med Fitness	High Fitness	Low Fitness	Med Fitness	High Fitness	Low Fitness	Med Fitness	High Fitness
GlycA (μmol/L)	377.24 (11.10)	360.40 (5.39)	341.77 (5.39)	397.87 (11.13)	387.21 (6.89)	372.97 (9.71)	440.58 (5.85)	412.96 (5.79)	366.88 (22.78)
LP-IR	26.26 (0.08)	23.71 (0.02)	20.61 (0.02)	33.16 (0.08)	37.44 (0.03)	32.10 (0.06)	55.51 (0.02)	50.49 (0.02)	42.77 (0.35)
LDL-P (nmol/L)	642.09 (54.83)	619.67 (25.71)	610.24 (25.69)	808.29 (54.97)	770.36 (33.56)	744.21 (47.83)	1024.60 (28.10)	857.27 (27.87)	735.31 (113.67)
Non-HDL-C (mg/dL)	96.00 (4.94)	99.80 (2.25)	96.58 (2.25)	111.92 (4.95)	114.36 (3.00)	108.11 (4.30)	127.99 (2.48)	114.85 (2.46)	105.85 (10.33)
8 th Grade									
GlycA (μmol/L)	364.98 (11.10)	362.42 (7.80)	358.62 (8.21)	390.55 (14.69)	376.18 (8.86)	360.56 (13.41)	415.15 (8.41)	398.60 (9.05)	376.11 (19.52)

<i>LP-IR</i>	28.13 (0.06)	29.14 (0.03)	28.16 (0.04)	40.69 (0.11)	33.91 (0.04)	35.47 (0.09)	45.39 (0.04)	41.78 (0.04)	38.07 (0.20)
<i>LDL-P</i> <i>(nmol/L)</i>	686.11 (38.31)	685.60 (26.89)	689.00 (28.37)	808.40 (51.11)	708.59 (30.87)	689.42 (46.57)	869.99 (29.33)	834.98 (31.60)	672.09 (67.91)
<i>Non-HDL-C</i> <i>(mg/dL)</i>	89.23 (3.31)	93.30 (2.34)	93.31 (2.47)	100.36 (4.37)	93.29 (2.68)	89.11 (3.98)	103.03 (2.53)	100.43 (2.72)	91.74 (5.76)

Low fitness=Q1 (6th Grade – Girls: 0-11 laps, Boys: 0-12 laps; 8th Grade – Girls: 1-12 laps, Boys: 1-17 laps),
Medium Fitness=Q2 & Q3 (6th Grade – Girls: 12-23 laps, Boys: 13-30 laps; 8th Grade – Girls: 13-26 laps, Boys: 18-44 laps)
High Fitness=Q4 (6th Grade – Girls: 24-57 laps, Boys: 31-75 laps; 8th Grade – Girls: 27-79 laps, Boys: 45-103 laps)
6th grade adjusted for 6th grade Tanner stage, race/ethnicity, SES category and intervention status
8th grade adjusted for 8th grade Tanner stage, race/ethnicity, SES category, intervention status and 6th grade value of the dependent variable (GlycA, LP-IR, LDL-P or Non-HDL-C)